J. Food and Dairy Sci., Mansoura Univ., Vol. 6 (5): 277 - 292, 2015

CHEMICAL AND BIOLOGICAL STUDIES ON FUNCTIONAL BISCUITS

Mona M. Khalil ; M. T. Shalaby ; Amaleka D. El-Dahshan and S. K. Saeed
Food Industries Dept - Fac. of Agric., Mansoura Univ. - Egypt

ABSTRACT

There is a global rise in the incidence of diseases like atherosclerosis and cardiovascular diseases. Therefore, this study was designed to examine the effect of adding chickpea and rice bran as a source of dietary fiber and active healthy components to prepare functional biscuits for lowering blood lipids. Functional biscuits were formulated by replacing wheat flour in biscuit formula by chickpeas or rice bran at 10, 20, 30 and 40% levels. Sensory evaluation revealed that successful replacement of biscuit samples were 10 and 20% chickpea (CP), 10 and 20% rice bran (RB) and (5% chickpea + 5% rice bran). Biscuits supplemented with 10 and 20% chickpea contained 1.13 and 1.27 times more protein than the control, while biscuits supplemented with 10 and 20% rice bran contained 1.95 and 2.91 times more of total dietary fibers, respectively than the control. The biological evaluation showed that hypercholesterolemic rats fed on diets containing 20% chickpea and 10% and 20% rice bran for 45 days significantly (p 0.05) reduced serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (vLDL), while high density lipoproteins (HDL) increased. Histopathological examination showed that feeding diets supplemented with different functional biscuits to the hypercholesterolemic rats lowered the degree of liver lesions . So it can be suggested that chickpea and rice bran can be used in preparation of functional biscuits for lipid lowering and liver functions improvement purpose.

Keywords : chickpea, rice bran, biscuits, chemical composition, lipid profile and liver function.

INTRODUCTION

Life style of people all over the world have changed in the last century due to income rise, increased leisure time and reduced physical activity. The new life style have considerable impact on health Venugopal B.V. (2009). Today foods are not intended to only satisfy hunger and provide necessary nutrients for humans but, also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. In this regard, functional foods play an outstanding role Hassan, et al., (2012).

The bakery industry is growing very fast and the products are increasingly becoming popular among all sections of people. Among ready-to-eat snacks, biscuits possess several attractive features including wider consumption base, relatively long shelf-life, more convenience and good eating quality (Hooda and Jood, 2005 and Iwegbue, 2012).

Rice bran is one of the most abundant co-products produced in the rice milling industry (FAOSTAT, 2013). Rice bran as a waste product of paddy milling contained protein, carbohydrate, dietary fiber, ash, fat, vitamin, mineral and natural antioxidant compounds (Chen et al., 2008 and Saenjum et al., 2012). Rice bran was supplemented in wheat flour to prepare fiber, mineral and protein enriched cookies for their allied health claims Sharif et al. (2009).

Chickpea (*Cicer arietinum* L.) is one of the most important grain-legume crops in the world, with a world production of 10.4 million ton (FAOSTAT 2011). Chickpeas are valuable source of calories, protein, minerals, fibers and minor component of potential health benefits Vega et al. (2010). Fibre-rich chickpea-based pulse diet has been shown to reduce the total plasma cholesterol levels in obese subjects Crujeiras et al. (2007). Feeding a chickpea diet to rats also resulted in a favorable plasma lipid profile Yang et al. (2007).

Hyperlipidemia, comprehensive hypercholesterolemia and hypertriglyceridemia, is a main danger factor for the expansion of cardiovascular diseases. The search for new drugs able to decrease and/ or to regularize serum cholesterol and triacylglycerol levels has obtained importance over the years, resulting in varied reports on significant activities of normal agents. Makni et al. (2008).

American heart association defined hyperlipidemia is a high level of fats in the blood. These fats, called lipids include cholesterol and triglycerides. There are different types of hyperlipidemia depending on which lipid levels are high in the blood Kishor et al.(2007). Thus, the objective of this study was to evaluate chemical and biological properties of Functional Biscuits.

MATERIALS AND METHODS

Materials :

wheat flour, chickpeas (*Cicer arietinum* L.), Sugar, salt, baking powder, fat, milk powder, Ammonium bicarbonate and vanillin was obtained from local market, El-Mansoura, Egypt.

Rice Bran was obtained from Bahrand Rice Mills, El-Mansoura, Egypt. All chemicals (analytical grade) were purchased from middle East company for medical materials, El- Gizah , Egypt. Male albino rats were obtained from Ophthalmology Research Institute, Giza, Egypt.

Methods :

Chickpeas preparation:

Chickpea seeds were cleaned and prepared by soaking for 6 hours at ambiant temperature with the replacement of soaking water every two hours, and then dried at 60 ^{o}C in an air drying oven (officine specializzate, GARBUIO essiccatoi, TREVISO, Italy) until drying, finally grinded into fine powder using a laboratory electronic mill(Brown model 2001 DL Germany at speed 2 for 3 min.) to pass through a 60 mesh sieve.

rice bran Preparation and stabilization:

Freshly milled rice bran was stabilized using the autoclave (Tuttnauer, 3850M, USA) for enzymes inhibition to prevent oxidation of fat in rice bran which characterized by higher fat .

Biscuits preparation :

Biscuit blends were processed using the method described by Islam et al. (2012). Blends of wheat flour with chickpeas and rice bran were prepared by substitution at 10, 20, 30 and 40% chickpea, 10, 20, 30 and 40% rice bran and 10, 20, 30 and 40% mixture (chickpea + rice bran). The ingredient such as wheat flour or wheat flour blends 100g, sugar 40g, fat 35g, salt 0.5g, skim milk powder 2g, water 15 ml, ammonium bicarbonate 1.5g and vanillin 5g as shown in Table (1). Were mixed properly to obtain uniform dough and the dough was allowed to relax for 15 minutes before rolling out. The dough was then kneaded and rolled to uniform thickness of 2 mm. The rolled out dough was allowed to relax for 5 minutes, Then the biscuits were cut out with round biscuits cutter of 5.5 cm diameter. Biscuits were placed on a greased or paper lined pans apart and the biscuit were allowed to rest on the pan for about 10 minutes and baked in an oven (Bakbar Versatile Bench Top Model E32, Germany) at 220 ^{o}C for 10-15 minutes, then cooled to ambient temperature and kept in polyethylene bags at room temperature until used.

Table (1) : formulation of biscuits

Ingredients	Blends					
	A	B1	B2	C1	C2	D
Wheat flour (g)	100	90	80	90	80	90
Chickpeas flour (g)	0	10	20	0	0	5
Rice Bran flour (g)	0	0	0	10	20	5
Sugar (g)	40	40	40	40	40	40
Fat (g)	35	35	35	35	35	35
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5
Skim Milk powder (g)	2	2	2	2	2	2
Water (ml)	15	15	15	15	15	15
Ammonium bicarbonate (g)	1.5	1.5	1.5	1.5	1.5	1.5
Vanillin (g)	5	5	5	5	5	5

Physical analysis of biscuit :-

Physical analysis of biscuits were carried out in the Agricultural Research Center, Giza, Egypt as following:

Volume, weight and thickness of biscuits were determined according to standard methods A.O.A.C (2007). Thickness of biscuits was measured by stacking six biscuits on top of one another and taking the average thickness of six biscuits in *cm*. and spread ratio % was calculated by dividing the average value of diameter / average value of biscuits thickness. Also, diameter was measured using (Boclase HL 474938, STECO, Germany).

Sensory evaluation of biscuit :-

Biscuit samples were evaluated organoleptically by a panel of ten panelists for appearance (10), color (15), thickness (15), crispiness (15), shrinkage (15), taste (15) and odor (15) as the method described by Smith (1972).

Analytical methods

Gross chemical analysis :

Moisture, crude protein, fat, crude fiber and ash were determined according to the method of the A.O.A.C (2007).

The percentage of carbohydrate were calculated by difference as the following equation :-

Carbohydrates % = 100 – (% crude fiber + % protein + % fat + % ash).

Biological assays:

Experimental animals:

Forty male albino rats weighting between 115-150 gm were used in this experiment Animals were housed in cages under normal healthy laboratory conditions (at 25 ^{o}C, 12hr light and 12hr dark) at Ophthalmology Research Institute, Giza, Egypt. The animals were fed on basal diet. Basal diet were presented in Table (2) salt and vitamin mixtures as described by A.O.A.C (2005) were shown in Tables (3 and 4) for 10 days as adaptation period prior to start of experiments.

Table (2): Composition of the basal diet:

Ingredient	Basal diet(g/100g)
Casein	10
Starch	70
Fat (Corn oil)	10
Mineral mixture	4
Vitamin mixture	1
Cellulose	5

A.O.A.C (2005).

Table (3): Composition of salt mixture.

Salt	Weight (g)
NaCl	139.300
K1	000.790
KH_2PO_4	389.000
$MgSO_4$	057.000
CaO_3	381.000
$FeSO_4.\ 7H_2O$	027.000
$MuSO_4.\ H_2O$	004.010
$ZnSO_4.\ 7H_2O$	000.548
$CuSO_4.\ 5H_2O$	000.470
$COCl_2.\ 6H_2O$	000.023

A.O.A.C (2005).

Table (4): Composition of vitamins mixture.

Vitamins	Weight(g)
Vitamin A palmitate (500.000 Iu / g)	00.80
Vitamin D3 acetate (1000 Iu / g)	01.00
Vitamin E acetate (500 Iu / g)	10.00
Menadione sodium Bisul-fate (62.5% menacione)	00.08
Thiamine HCl	00.60
Riboflavin	00.60
Pyridoxine HCl	00.07
Nicotinic acid	03.00
Calcium pantothenate	01.60
Folic acid	00.20
Biotin , 1%	02.00
Cyano Cobalamine 0.01%	01.00
Sucrose	978.42
Total	1000

A.O.A.C (2005).

Experimental design:

This study aimed to compare the effect of the eight different types of diets. After feeding on basal diet for 10 days (adaptation period). Five rats were kept as a control which fed basal diet during the experiment period G1 (negative control). The other groups (from G2 to G8) were fed with high-fat diet with 1% cholesterol. All groups of rats were fed the experimental diets for 45 days as follows:

- Group (1) rats were fed on a basal diet (negative control).
- Group(2) rats were fed on hyperlipedemic and hypercholesterolemic diet (positive control).
- Group (3) hyperlipedemic rats were fed on a control Biscuit.
- Group (4) hyperlipedemic rats were fed on Biscuit containing 10% Rice Bran.
- Group (5) hyperlipedemic rats were fed on Biscuit containing 20% Rice Bran.
- Group (6) hyperlipedemic rats were fed on Biscuit containing 10% chickpeas.
- Group (7) hyperlipedemic rats were fed on Biscuit containing 20% chickpeas.
- Group (8) hyperlipedemic rats were fed on Biscuit containing 5% chickpeas and 5% Rice Bran.

Blood Sampling: blood samples were taken at zero time (after feeding hyperlipedemic diet for 3 weeks) and at the End of experiment, Then centrifuged to obtain serum and stored at (-20ºC) until assayed.

Biochemical analysis: Serum total cholesterol was determined according to the method described by Allain, et al. (1974). Triglycerides were determined according to the method described by Fossati and Principe (1982). Serum HDL-cholesterol was determined by the method of Lopez-Virell et al., (1977). Serum Low Density Lipoprotein (LDL-cholesterol) was determined by the method of Wieland and Seidel (1982). Triglycerides were determined according to the method described by Fossati and Principe (1982).

Histopathological techniques:

Samples were collected from liver for all experimental groups and fixed in neutral buffered formalin 10% for 24 hrs. The samples were routinely processed, Paraffin embedded, and sectioned at 4 – 6 micron thickness. The prepared slides were stained by hemetoxylin and eosin Bancroft and Stevens (1996). Examinations were performed at the Histology Laboratory of Faculty of Veterinary Medicine, Cairo University according to the methods of Drury and Wallington (1980).

Sections were examined by light microscopy. The histopathological results of the different groups were photographed.

Frozen specimens from liver were cut into section using cryostat, and then stained with oil red stain.

statistical analysis: Data was analyzed using analysis of variance. Significance was used at $P.<0.05$. (ANOVA) to determine significant difference among the various samples. Data were analyzed by using means of Statistical Analysis System SAS, (1996).

RESULTS AND DISCUSSION

Chemical composition of raw materials:

Wheat flour , Rice bran and chickpea (*Cicer arietinum* L.) were analyzed for moisture, protein, ash, ether extract, crude fiber and carbohydrates. The obtained results are shown in Table (5). Wheat flour contained 0.6% ash, 9.064% protein, 1.5% ether extract, 0.87% crude fiber and 87.966% available carbohydrates. Analysis of dietary fiber sources for ash showed that rice bran flour had the highest ash content (8.141%) followed by chickpea (3.564%). Concerning protein content, chickpea was the highest protein content (22.212%). Rice bran had a protein content of (12.800%),

For fat content (ether extract), rice bran contained the highest content of ether extract (17.761%). On the other hand, chickpea had a ether extract content of (6.0%). Crude fiber content of dietary fiber sources could be arranged in the following descending order: 9.27% and 3.18% for rice bran and chickpea, respectively. These results are in agreement with those obtained by (Samli et.al., (2006); Wanyo et al., (2009) and Abou Arab et al., (2010)). The results showed that rice bran the lowest level of carbohydrates (52.028%). These results could be due to the highest content of protein, fat and ash, the carbohydrate content of chickpeas was (65.044%). These results are in agreement with those reported by (Hassan et al., (2012) and Saleh et al., (2012)).

Table (5): Chemical composition of raw materials used in making biscuit (on dry weight basis).

Sample No.	Moisture %	Protein %	Fat %	Crude Fiber %	Ash %	Total Carbohydrates %
Wheat flour 72% (WF)	11.742	9.064	1.5	0.87	0.6	87.966
Rice Bran (RB)	11.588	12.800	17.761	9.27	8.141	52.028
Chickpea (CP)	10.250	22.212	6.0	3.18	3.564	65.044

Sensory evaluation and Physical properties of biscuit :

Average sensory panel scores of appearance, color, Thickness, Crispiness, Shrinkage, Taste, Odor and Overall acceptability for biscuits formulas contained wheat flour and its mixtures with rice bran and chickpea are tabulated in Table (6) Control sample (100% wheat flour) showed the best overall acceptability (87.83%) followed by the sample No (1) which contain 90% wheat flour + 10% chickpea (83.83%). Samples (4) contain 80% wheat flour + 20% chickpea (82.42%). Samples (2) which contain 90% wheat flour + 10% rice bran (81.75%). Samples (5) which contain 80% wheat flour + 20% rice bran (81.58%) and sample No (3) which contain 90% wheat flour + 5% chickpeas + 5% rice bran (81.17%) while the overall acceptability for the rest samples ranged between 69.75% to 80.67%, these variations are actually related to levels of raw materials.

6-

Physical measurements of biscuit samples :

Biscuits were subjected to physical measurements including weight, volume, specific volume, diameter, thickness and spread factor. Measurements of flour substituted biscuits at various levels are shown in Table (7). Results indicated that specific volume of wheat flour substituted biscuits (which relates with good crispness and texture) were lower than control biscuit. A negative relationship could be noticed between flour substitution level and biscuits specific volume. Biscuits specific volume 2.57cm³/gm and ranged from 1.89 to 2.18cm³/gm for biscuit samples containing flour substitution. Such decrease in biscuits specific volume could be attributed to good humactancy and water holding of other flours. For other measurements, it could be noticed that biscuits spread ratio decreased as wheat flour replacement level increased. Biscuits spread ratio was 7.5 for control sample, it was the highest value of spread ratio in all biscuit samples. While the lowest spread ratio was 5.73 in biscuit samples making substitute wheat flour with 5% chickpea + 5% rice bran. The changes in baking properties may be due to the changes in the quality and quantity of protein with the added ingredients and also attributed to gas retention of dough during baking process Sai and Ras(1997).

Table (7): Physical properties of biscuits produced from wheat flour supplemented with different levels of chickpeas and/or rice bran.

Samples No.			Specific volume			Spread ratio		
			Weight (gm)	Volume (cm³)	Specific volume (cm³ / gm)	Diameter Width) (cm)	Thickness (cm)	Spread ratio W/T
Con.		100% wheat flour	50.6	130	2.57	6.0	0.8	7.5
10% wheat flour substitution	1	90% WF +10% CP	63.45	120	1.89	5.5	0.9	6.11
	2	90% WF +10% RB	75.59	160	2.12	6.0	0.95	6.32
	3	90% WF + 5% CP + 5% RB	60.49	130	2.15	5.5	0.96	5.73
20% wheat flour substitution	4	80% WF + 20% CP	64.81	125	1.93	5.5	0.9	6.11
	5	80% WF + 20% RB	71.05	155	2.18	6.35	0.92	6.90
	6	80% WF + 10% CP + 10% RB	60.82	132	2.17	5.6	0.96	5.83
30% wheat flour substitution	7	70% WF +30% CP	64.72	124	1.91	5.8	0.93	6.23
	8	70% WF+ 30% RB	70.5	153	2.17	6.37	0.91	7
	9	70% WF + 15% CP + 15% RB	60.73	131	2.15	5.6	0.95	5.89
40% wheat flour substitution	10	60% WF + 40% CP	64.91	126	1.94	5.7	0.92	6.19
	11	60% WF + 40% RB	70.35	151	2.14	6.38	0.90	7.08
	12	60% WF + 20% CP + 20% RB	61.02	129	2.11	5.9	0.97	6.08

WF= wheat flour. . CP= Chickpea. RB= Rice bran

So, according to obtained results of above analysis of sensoncal and physical properties of different biscuit mixtures samples, it was decided to select samples, No 1, 2,3,4 and 5 beside control due to their high acceptability and good physical properties to continue different analysis and evaluations.

Chemical components of biscuit samples (on dry weigh basis):

The chemical composition of biscuit produced from wheat flour(WF) 72% extraction with chickpeas (CP) and rice bran (RB) blends are presented in Table (8) .

Two levels (10 and 20%) chickpeas and rice bran were used to substitute wheat flour in biscuit making. biscuit samples and control were analyzed for their chemical analysis, like moisture, protein, ash, fat, crude fiber and total carbohydrate content. The obtained results are shown in Table (8). The moisture content was found to be 5.87% in biscuit sample (control). It is clear that it increased by 6.5, 6.7, 6.0, 6.2 and 5.98% in chickpea biscuit at replacement level 10%, chickpea biscuit at replacement level 20%, rice bran biscuit at replacement level 10%, rice bran biscuit at replacement level 20% and mixture biscuit at replacement levels 10% (5% chickpea and 5% rice bran), respectively compared to control. Fat content of control sample was 18.23%. It was 18.49, 18.76, 19.14, 20.06 and 18.81% for chickpea biscuit at replacement level 10%, chickpea biscuit at replacement level 20%, rice bran biscuit at replacement level 10%, rice bran biscuit at replacement level 20% and mixture biscuit at replacement levels 10% (5% chickpea and 5% rice bran), respectively, compared to control sample, analysis of biscuit samples for fat showed that rice bran biscuit at replacement level 20% had the highest fat content (20.06%) followed by rice bran biscuit at replacement level 10% (19.14%). Crude fiber content data in Table (8) showed that the rice bran biscuits with replacement level 20% contained the highest content of crude fiber (1.40%) followed by the rice bran biscuits with replacement level 10% contained (0.94%) crude fiber. The value of crude fiber content of control sample was (0.48%). In addition, it could be noticed that other replacement of chickpeas and mixture leaded to an increase in fiber content was 0.61, 0.74 and 0.77% compared with control sample content was 0.48%. Ash content was found to be 1.43%, in control biscuits, It was 1.66, 1.88, 1.85, 2.26 and 1.76% for in chickpea biscuit at replacement level 10%, chickpea biscuit at replacement level 20%, rice bran biscuit at replacement level 10%, rice bran biscuit at replacement level 20% and mixture biscuit at replacement levels 10% (5% chickpea and 5% rice bran), respectively compared to control.

Table (8): Chemical composition of biscuit produced from wheat flour 72% ext., and its mixtures (on dry weigh basis).

Sample code		Moisture %	Protein %	Fat %	Crude Fiber %	Ash %	Carbohydrates %
Cont.	100% w heat flour	5.87	5.57	18.23	0.48	1.43	74.30
1	90% WF + 10% CP	6.5	6.34	18.49	0.61	1.66	72.9
2	90% WF + 10% RB	6.0	5.78	19.14	0.94	1.85	72.29
3	90% WF + 5% CP + 5% RB	5.98	6.06	18.81	0.77	1.76	72.6
4	80% WF + 20% CP	6.7	7.12	18.76	0.74	1.88	71.5
5	80% WF + 20% RB	6.2	5.98	20.06	1.40	2.26	70.3

WF= wheat flour. CP= chickpea. RB= rice bran

Biological evaluation

Hypocholesterolemic effects of different diets :

Data in Table (9): illustrated the mean values of serum triglycerides, total cholesterol, low density lipoprotein (LDL), very low density lipoprotein (vLDL) and high density lipoprotein (HDL) of rats fed different diets containing biscuits with different levels of wheat flour substitute. With regard to serum total cholesterol, results in Table (9) disclose that, all mean values of total cholesterol for rat groups fed diets (3), (4), (5), (6), (7) and (8) decreased (from zero to end time) than that of group 4, 5, 6 and group 7. Significant differences were obtained between the values of cholesterol for all tested rat groups. Total cholesterol values were 175.8, 171.0, 174.5 and 169.8 mg/dl for groups 4, 5, 6 and 7, respectively. Regarding serum triglycerides, data show that triglycerides for rats fed on different tested diets were significantly decreased at the end of experimental time for rat groups fed diets (1),(3), (4), (5), (6), (7) and (8). Rat fed on diet containing biscuits with 20% rice bran (group 7) showed the highest percentage of decrease being (20% compared with the same group at zero time) followed in descending order by group 5 (19.19 % compared with the same group at zero time) then group 6 (17.08% compared with the same group at zero time), compared to negative control (1.81% compared with the same group at zero time). Triglycerides value of rats group fed on group (1) being 72 mg/dl which was the lowest record, followed by group 7(77.33 mg/dl), group 6 (77.67 mg/dl), group 5 (80.0 mg/dl), group 8 (84.33mg/dl), group 4 (88.33 mg/dl) then, those recorded for rat groups fed on group 2 (90.67 mg/dl) . Data also show that substitution of wheat flour with chickpea and rice bran reduced low density lipoprotein (LDL) by different ratios depending on the kind of substitution. The LDL values were 66.67, 62.33, 63.00, 58.67, 60.67 and 68.67 mg/dl for rates fed on groups 3, 4, 5, 6, 7 and 8, respectively. significant differences in LDL values were noticed between groups 1, 2, 3 and 4 and significant differences were noticed between LDL values of rat groups 7 and 8. While there was no significant difference between groups 4, 5, 6 and 7.

From Table (9) noticed the vLDL values at the end of experimental time were 14.73, 20.87, 18.13, 17.67, 16, 15.53, 15.87 and 16.27 mg/dl for groups 1, 2, 3, 4, 5, 6, 7 and 8, respectively.

The obtained results in the same Table (9) indicate that the mean values of high density lipoprotein (HDL) of rats fed on all experimental diets except positive group (2) showed in this group decrase in value of HDL (from zero to the end of experimental time) 8.73%. On another hand, groups 2,3,4,5,6,7 and group 8 showed high percentage of increase in values of HDL (from zero to the end of experimental time) compared with negative control G1, being 27.83 % for group(7), 24.50 % for group (5), 23.44 % for group (6), 21.99 % for group (8) and 15.2 % for group (3), in comparison with that fed on negative control (1), 0.5%. The HDL values at the end of experimental time were 48, 31.33, 35.67, 41.33, 40.33, 41.33 and 37 mg/dl for groups 1, 2, 3, 4, 5, 6, 7 and 8, respectively.

As Lee et al., (2008) found that the decrease of LDL-cholesterol prevent the heart from coronary disease, it logically that substitution of wheat flour with legumes flour is the best because it record the highest value of reducing.

Yang et al. (2007) found that several pro-atherogenic factors, including triacylglycerol (TG), low density lipoprotein LDL-C, and ratio LDL-C:HDL-C, decreased with consuming chickpea based diet.

Phytosterols in chickpeas along with other factors e.g. isoflavones, oligosaccharides, reduces the LDL-C levels in blood by inhibiting the intestinal absorption of cholesterol due to it parity in their chemical structure for cholesterol that way potentially decrease the risk of CHD Wood and Grusak (2007)

9-

Histopathological investigation :

Microscopically, examination of liver on negative control group (1), revealed almost close histological picture. Negative control group (G1) showed the normal histological structure of hepatic lobule Fig.,1(A). Untreated positive control group (G2) showed hydropic degeneration change in the hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration Fig.,1 (B).

Meanwhile, more extensive lesions were seen in case of rats fed on diet contain biscuits made from wheat flour group (3) Fig.2(A), in comparison to those normal histological criteria of the hepatic tissue of rats fed on basal diet (group 1). These extensive lesions in group 3 were manifested by fatty change of hepatocytes, congestion of hepatic sinusoids and slight hydropic degeneration of hepatocytes. On regard to the microscopic examination for the liver of groups 4, 5, 6, 7 and group 8 Fig.2(B and F) rats showed no histopathological changes except kupffer cells activation and showed slight hydropic degeneration of hepatocytes, respectively .

So above obtained results cleared that feed on hypercholesterolemia lead to an extensive alteration that mostly resulted clinically into failure in liver and appears low effect in the affected animals fed on biscuits supplemented with chickpea and rice bran. Also, it was concluded that the used chickpea and rice bran led to some but not complete antagonistic or protective effects against the hypercholesterolemia , as a moderate changes were seen in the studied organs of these cases

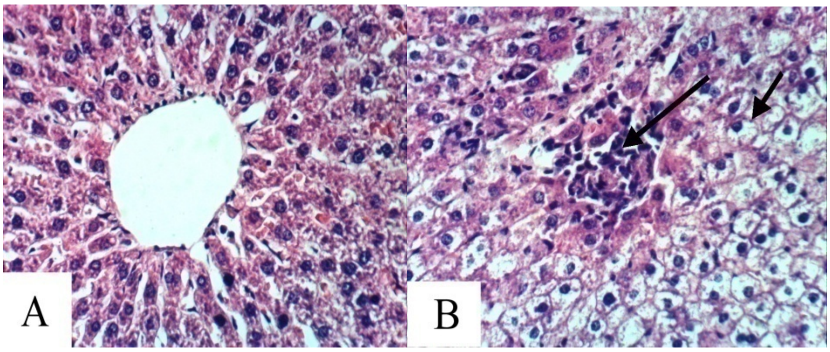

Fig.1 (A and D) A panel showing photomicrograph of a paraffin sections in the liver of control negative and control positive rats :

A- Section in control negative rat liver showing the normal histological structure of hepatic lobule. (H , E X 400)

B- Section in control positive rat liver showing hydropic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration. (H , E X 400)

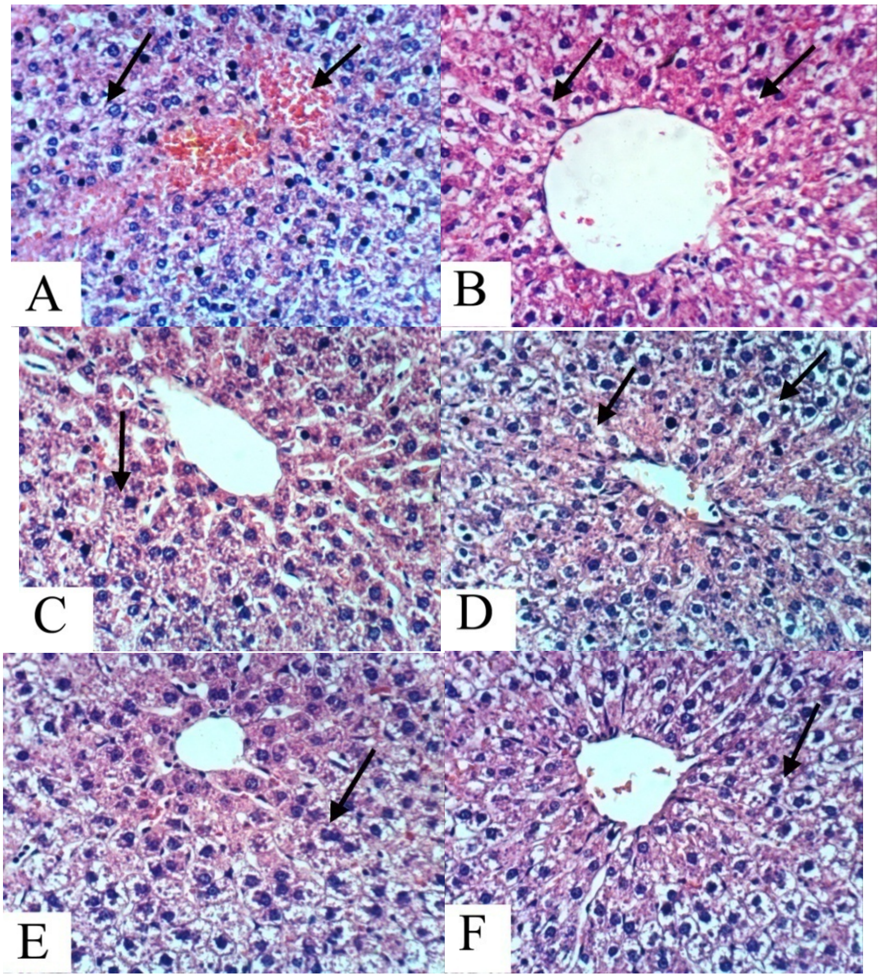

Fig.2 (A, B, C, D, E and F) A panel showing photomicrograph of a paraffin sections in the liver of hypercholesterolemia, treated rats :

A- Section liver of rat in Group (3) liver showing congestion of central vein and hydropic degeneration of hepatocytes (H , E X 400).

B- Section liver of rat in (4) liver showing slight hydropic degeneration of hepatocytes . (H , E X 400)

C- Section liver of rat in Group (5) liver congestion of central vein and hydropic degeneration of hepatocytes. (H , E X 400).

D- Section liver of rat in Group (6) liver showing congestion of central vein and hydropic degeneration of hepatocytes. (H , E X 400)

E- Section liver of rat in Group (7) liver showing slight hydropic degeneration of hepatocytes. (H , E X 400)

F- Section liver of rat in Group (8) liver showing congestion of central vein and hydropic degeneration of hepatocytes. (H , E X 400)

REFERENCES

A.O.A.C. (2005). Official Method of Analysis. Association of Official Analytical Chemists Washington DC, USA.

A.O.A.C. (2007). Official Method of Analysis. (16^{th} End) Association of Official Analytical Chemists Washington DC, USA.

Abou Arab. E. A.; I. M. F. Helmy and G. F. Bareh, (2010). National Evaluation and functional properties of chickpea (Cicer arietinum L.) flour and the improvement of spaghetti produced from its. Journal of American scince, 6(1).

Allain, C.C.; L.S., Poon; C.S.G., Chan; W. Richmond and P.C., Fu (1974). Enzymatic Determination of Total serum cholesterol Clin Chem, 20:4, 470-475.

Bancroft, J.D. and A. Stevens, (1996). Theory and practice of histological techniques. 4 edn, Churchill th livingstone, New York.

Chen, C.R.; C.H. Wang; L.Y. Wang; Z.H. Hong; S.H. Chen and W.J. Ho (2008). Supercritical-carbon dioxide extraction and deacidification of rice bran oil. Journal Supercritical Fluids 45: 322-331.

Crujeiras A.B.; D. Parra; I. Abete and J.A. Martinez, (2007). A hypocaloric diet enriched in legumes specifically mitigates lipid peroxidation in obese subjects. Free Radical Research 41, 498-506.

Drury, A. A. and E. A. Wallington, (1980): Carleton's histological technique.5th Ed.,Oxford University press, New York, Toronto.

FAOSTAT.(2011).Statisticaldatabase.Availableat:http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID ¼ 567#ancor. Last accessed 28 October.

FAOSTAT. (2013). FAO Statistics Division. http://fao.org.

Fossati P, and Principe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem.28: 2077-2080.

Hassan A. A.; N. M. Rasmy; M. I. Foda and W. K. Bahgaat, (2012). Production of functional Biscuits for lowering Blood lipids. Wourld journal of dairy and sciences, 7(1) : 01-20.

Hooda, S. and S. Jood 2005. Organoleptic and nutritional evaluation of heat biscuits supplemented with untreated and treated fenugreek flour. Food Chemistry 90:427–435.

Islam, M. Z.; M. L. J. Taneya; M. Shams-Ud-Din; M. Syduzzaman and M. M. Hoque, (2012). Physicochemical and Functional Properties of Brown Rice (Oryza sativa) and Wheat (Triticum aestivum) Flour and Quality of Composite Biscuit Made Thereof. A Scien. J. of Krishi Foundation 10 (2): 20-28.

Iwegbue, C.M.A., (2012). Metal contents in some brands of biscuits consumed in southern Nigeria. American Journal of Food Technology 7:160-167.

Kishor J.S; M.K. Kathivarin; S. Rahul and J. chamanal (2007). The biology and chemistry of hyperlipidemia.Bioorganic And Medicinal Chemistry, 15,4674-4699.

Lee, Y.P.; I.B. pudey and J.M. Hodgson, (2008). Protein, fiber and blood pressue: Phamacology and Physology, 35:PP473-476.

Lopez-Virella, M. F.; P. Stone; S. Ellis and J. A. Colwell, (1977). Cholesterol determination in high density lipoproteins separated by three different methods. Clin Chem,23:882-884.

Makni, M.; H. Fetoui; N. K. Gargouri; El M. Garoui; H. Jaber; J. Makni; T. Boudawara and N. Zeghal, (2008). Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in n-3 and n-6 fatty acids in hypercholesterolemic rats. Food and Chemical Toxicol., 46: 3714-3720.

Saenjum, C.; C. Chaiyasut; S. Chansakaow; M. Suttajit and B. Sirithunyalug, (2012). Antioxidant and anti-inflammatory activities of gamma-oryzanol rich extracts from Thai purple rice bran. Journal Medical Plants Research 6: 1070-1077.

Sai, M. R. and Ras, P. H. (1997). Effect of mixing period and additives on ricological characteristics of dough and quality of biscuits. J. Cereal Sci. , 25:197-206.

Saleh. A. M.; A. Salama; S.H. Bedeir and E.I.Abd-Elazim, (2012). Effect of partial substitution of wheat flour with either defatted soybean or chickpea flour at different ratios on rheological and physical properties of dough, and quality characteristics of biscuit. Journal of Applied sciences research, 8(12) : 5806-5817.

Samli H.E.; N. Senkoylu; H. Akyurek and A. Agma, (2006). Using rice bran in laying hen diets. Journal of Central European Agriculture 7: (1) 137-140.

SAS, (1996). Statistical Analysis System, SAS user's guide states statistics. SAS Institute INC. Editors, Cary, NC. USA.

Sharif M.K.; M.S. Butt; F.M. Anjum and H. Nawaz, (2009). Preparation of Fiber and Mineral Enriched Defatted Rice Bran Supplemented Cookies. Pakistan Journal of Nutrition 8 (5): 571-577.

Smith W.H., (1972). Biscuits crackers and cookies ; Technology, preparation and management. Applied Science publisher, London.

Venugopal B.V., (2009). Functional foods: An overview, Marine Products for Healthcare- Functional and Bioactive Nutraceutical Compounds from the Ocean, CRC Press, Boca Raton, pp. 1-22.

Vega C.R.; G.Loarca, and B.D. Oomah, (2010). Minor components of pulses and their potential impact on human health. Food Res Int. 43: 461–482.

Wanyo P.; C. Chomnawang and S. Siriamornpun, (2009). Substitution of wheat flour with rice flour and rice bran in flake products: Effects on chemical, physical and Antioxidant properties. World Applied sciences journal, 7(1):49-56.

Wieland, H., and D. Seidel. (1982). Improved assessment of plasma lipoprotein patterns. IV. Simple preparation of a lyophilized control serum containing intact human plasma lipoproteins. Clin. Chem. 28 1335-1337.

Wood J.A., and M.A. Grusak, (2007). Nutritional value of chickpea. In Chickpea breeding and management. pp. 101-142 [SS Yadav, R Redden, W Chen and B Sharma, editors]. Wallingford, UK: CAB International.
Yang Y.; L. Zhou; Y. Gu; Y. Zhang; J. Tang; F. Li; W. Shang; B. Jiang; X. Yue and M. Chen, (2007). Dietary chickpea reverse visceral adiposity, dyslipidaemia and insulin resistance in rats induced by a chronic high-fat diet. Br J Nutr 98, 720-726.

دراسات كيميائية وبيولوجية على البسكويت الوظيفي

منى محمود خليل ، محمد طه شلبي ، اماليكا درويش الدهشان و سامي خضر سعيد
قسم الصناعات الغذائية – كلية الزراعة – جامعة المنصورة – مصر.

نظراً للارتفاع العالمي في معدلات الإصابة بالأمراض مثل أمراض تصلب الشرايين وأمراض القلب والأوعية الدموية، فأن الأغذية الوظيفية تلعب دورا بارزا . لذلك تم تصميم هذه الدراسة لغرض دراسة تأثير إضافة حمص الشام ونخالة الأرز (كمصدر للألياف الغذائية والمكونات النشطة صحياً) على الخصائص الكيميائية والبيولوجية للبسكويت الوظيفي .

حيث كشف التقييم الحسي أن عينات البسكويت المصنوع من استبدال دقيق القمح مع حمص الشام بنسبة ١٠ و٢٠% ، نخالة الأرز ١٠ و٢٠% و(٥% نخالة الأرز +٥% حمص الشام) كانت الأكثر قبولا بعد عينة التحكيم . أما التحليل الكيميائي للبسكويت فقد كشف بان البسكويت المصنوع من استبدال دقيق القمح مع حمص الشام بنسبة ١٠% و ٢٠% كانت تحتوي على بروتين بنسبة ١٣,٨٢% و ٢٧,٨٢% ، على التوالي أكثر من عينة التحكيم. في حين ان البسكويت المصنوع من استبدال دقيق القمح مع نخالة الأرز بنسبة ١٠% و٢٠% كانت تحتوي على ألياف غذائية بنسبة ٠,٩٥% و١.٩١%، على التوالي أكثر من عينة التحكيم.

كشف التقييم البيولوجي إن الفئران التي تم تغذيتها على وجبات مرتفعة الكوليسترول لمدة ٤٥ يوم المجموعة ٦، ٧ و٨ كانت الأكثر تأثيرا حيث كانت هناك فروق معنوية عند (P 0.05) وخفضت كولسترول الدم، الكليسريدات الثلاثية ،البروتينات الدهنية منخفضة الكثافة ، البروتينات الدهنية منخفضة الكثافة جداً، في حين نلاحظ زيادة البروتينات الدهنية مرتفعة الكثافة

وأظهر فحص الأنسجة لكبد الفئران التي تم تغذيتها على الوجبات الغذائية المحتوية على البسكويت انخفاض قليل في معدل الإصابة مقارنة مع كبد الفئران في المجموعة رقم ٢ التي تم تغذيتها على الوجبات مرتفعة الكولسترول حيث كانت إصابة أنسجة الكبد فيها بشكل اكبر .

توصي الدراسة : باستخدام حمص الشام ونخالة الأرز في تجهيز وتصنيع البسكويت لخفض ليبيدات الدم وتحسين وظائف الكبد .

الكلمات الدالة : حمص الشام ، نخالة الأرز، البسكويت، التركيب الكيميائي، ليبيدات الدم، وظائف الكبد.